

Agarose embedding

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Updated date: Jan 7, 2023



An abbreviated version of this protocol was published in eLIFE in Feb 2020

Revisiting the role of Dcc in visual system development with a novel eye clearing method

DOI: 10.7554/eLife.51275

Detailed protocol

Dear Rachel,

In order to avoid any air bubble formation during eyeball embedding, several steps are important.

1) you have to avoid air bubbles in the embedding medium. See below the detailed protocol.

Embedding medium: **7.5% gelatin, 10% saccharose in 0.12 M Phosphate buffer**

Protocol for 1 L

- Dilute 100 g sucrose in 500 ml of 0.24 M Phosphate Buffer + 300 ml of mQ H₂O in a 1 L beaker.

- Melt 75 g gelatin (from porcine skin type A) in this sucrose solution by heating **slightly** on a hot plate stirrer. Heating too much will turn the solution into caramel. Everything should be well dissolved.

- QSP 1 L with mQ H₂O.

- Aliquot in PolyPropylene tubes with screw caps and store at -20°C.

2) Thaw your embedding medium aliquot in a water bath at around 40°C. Shake my inverting gently to ensure that the solution is homogeneous and bubble free.

3) From this step, use 3 ml plastic transfer pipette to manipulate the embedding medium.

4) Cover the bottom of a weighing boat (46 mm square) with embedding medium and let it gel. To accelerate the process, you can put the weighing boat in the fridge or on ice.

5) Lay the eyeball on the gelled embedding medium and cover it gently with liquid embedding medium. Wait until it is completely gelled before cutting off the excess embedding medium.

Best,

Kim

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Vigouroux, R. , Chédotal, A. and Nguyen-Ba-Charvet, K. (2023). Agarose embedding. Bio-protocol Preprint. bio-protocol.org/prep2107.
2. Vigouroux, R. J., Cesar, Q., Chédotal, A. and Nguyen-Ba-Charvet, K. T.(2020). Revisiting the role of Dcc in visual system development with a novel eye clearing method. eLIFE. DOI: [10.7554/eLife.51275](https://doi.org/10.7554/eLife.51275)

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